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Title: **New Uses of Lp-PLA2 in Combination
to Assess Coronary Risk**

DECLARATION BY Robert L. Wolfert, Ph.D.

I, Robert L. Wolfert, Ph.D., hereby declare:

1. I was awarded a B.A. in Chemistry and Biology from Cornell University in 1975 and a Ph.D. in Biochemistry and Immunology from Tufts University School of Medicine in 1981. After obtaining these degrees I served two Post-Doctoral Fellowships at the Scripps Clinic and Research Foundation in La Jolla, California in the Department of Immunopathology. The first was from 1981-1982 as a Leukemia Society Fellow and the second was from 1983-1984 as a NIH Research Fellow. From April 1984 to April 1999, I served in various capacities at Hybritech Incorporated, a diagnostics company, where I most recently held the position of Director of Immunodiagnostics Research. While at Hybritech I was involved in the development of the Tandem®-PSA assay which is routinely used today for prostate cancer screening. From May 1999 to October 2000, I was Vice President, Research and Development of Atairgin Technologies. In November 2000 I joined diaDexus, Inc. as Vice President, Diagnostics and currently serve as the Chief Scientific Officer and Executive Vice President, Research and Development. While at diaDexus I have directed the development, validation and FDA approval of

the PLAC® Test, an immunoassay for the measurement of Lp-PLA2 to aid in assessing a patient's susceptibility to CHD and stroke.

2. As Chief Scientific Officer at diaDexus and a co-inventor of the above referenced patent application I am familiar with the teachings of the patent application, the field of clinical diagnostics and statistical analysis utilized in developing diagnostic methods and products.

3. At the request of our attorneys, I have reviewed the Office Actions mailed April 15, 2008 and October 17, 2008 in this patent application. I also reviewed Packard et al. (NEJM 2000 343:1148-1155) and Rao et al. (US 2003/0120134). I understand that the Examiner has rejected claims to a method for assessing risk of Coronary Vascular Disease (CVD) in a patient which comprises measuring levels of both Lipoprotein Associated Phospholipase A2 (Lp-PLA2) and C-reactive protein (CRP) or Low Density Lipoprotein Cholesterol (LDL) in the patient, analyzing a risk associated with the level of CRP or LDL and a risk associated with the level of Lp-PLA2, and using the combined risks to assess the risk of CVD in the patient as being anticipated by Packard et al. (NEJM 2000 343:1148-1155) and obvious over Packard et al. (NEJM 2000 343:1148-1155) and further in view of Rao et al. (US 2003/0120134).

4. I disagree with the Examiner's position that Packard et al. teaches the invention claimed in this patent application. Packard et al. does not teach using the combined risks of CRP and Lp-PLA2 to assess the risk of cardiovascular disease in a patient. At the outset, I note that Packard et al. is discussed under the Clinical Review section of the patent application at page 6. The application states that Packard et al. teach "The association of Lp-PLA2 with CHD was independent of traditional risk factors such as LDL-cholesterol and other variables." This is true. The results in Table 5 of Packard are from multivariate analysis using different models (model 1, model 2 and variants of model 2) to determine how each marker (e.g. Lp-PLA2 or CRP) individually affects the risk of a coronary event. The statistical models employed by Packard show the individual relative risk for each marker by adjusting for the effects of all the other markers in the model. This adjustment is not a combination of the risk of each marker. The models are used to refine or determine how predictive each marker may be separately

in light of the predictive value of the other markers in the model. Packard never combines the markers or determines the combined risk of markers. Table 5 does not show a combination of Lp-PLA2 and CRP or a risk of CVD for such combination. Nor does Packard's finding in the discussion on page 1152 that CRP and Lp-PLA2 are both independent predictors of risk of coronary heart disease teach any combination of the risks of CRP and Lp-PLA2 to assess risk of coronary heart disease.

5. In contrast to Packard et al., the patent application teaches combining the markers Lp-PLA2 and CRP and then shows the effects of such a combination on risk of cardiovascular disease in a patient. Figure 7 of the application is a graphical representation of the data presented in Table 4.11 on page 32. These data show how the combination of markers Lp-PLA2 and CRP affect risk of CVD in a patient. Patients with a high level of either CRP or Lp-PLA2 have moderately elevated risk of CVD as compared to patients who have low levels of both markers. However, patients have a statistically significant elevated risk of CVD if they have high levels of both CRP and Lp-PLA2. This holds true in further examples where the combined risks of CRP and Lp-PLA2 are evaluated in patients with low LDL cholesterol levels and when the risks of CRP and Lp-PLA2 are combined from grouping patients by low, medium and high CRP and Lp-PLA2 levels. In fact, as shown in Figure 9, and the data in Table 4.11 at page 33, the risk for a patient with low LDL cholesterol who has the highest levels of both Lp-PLA2 and CRP is over three times higher than a patient with low cholesterol who has the highest level of either Lp-PLA2 or CRP (relative risks: $4.22/1.35=3.13$ and $4.22/1.2=3.52$). The patent application therefore describes how to combine the risks of Lp-PLA2 and CRP to assess the risk of cardiovascular disease in a patient.

6. Beyond teaching combining risks of Lp-PLA2 and CRP to assess the risk of CVD in a patient, the patent application has another difference over Packard et al. The patent application teaches risk of CVD using risk ratios which take into account that a CVD event occurred in a patient and the time to such event. Packard et al. teaches relative risks which only take into account that a CVD event occurred in a patient. By determining a patient's risk of CVD using risk ratios the patent application for the first time teaches combining Lp-PLA2 and CRP risks to assess risk of a CVD event and the time in the future that

such a CVD event may occur. Figure 1 shows the proportion of patients in different combined CRP and Lp-PLA2 risk groups without a CVD event over time. Figure 1 show that patients with high levels of both Lp-PLA2 and CRP had a statistically significant decrease in time to a CVD event compared to patients with low levels of both Lp-PLA2 and CRP and compared to patients with a high level of either Lp-PLA2 or CRP. Thus the patent application teaches the combination of Lp-PLA2 and CRP risks to assess the risk of CVD in patients within a timeframe. Packard et al. does not teach such a combination of Lp-PLA2 and CRP risks.

7. I also disagree with the Examiner's position that it would have been obvious in light of the teaching of Packard et al. and further in light of Rao et al. to combine the risks of Lp-PLA2 and CRP and the ATP III guidelines to assess risk of coronary heart disease. Subsequent to the publication of Packard et al., Blake et al. (*J Am Coll Cardiol* 2001 35(5):1302-6), discussed on page 7 of the patent application and attached hereto, reported that Lp-PLA2 was not a significant predictor of future cardiovascular risk when adjusted for traditional cardiovascular risk factors. Blake et al. did find that CRP was a significant predictor of cardiovascular risk. Thus, I disagree with the Examiner that it would have been obvious to combine the risks Lp-PLA2 and CRP or the risks of Lp-PLA2 and CRP and the ATP III guidelines when at the time of the invention there were conflicting reports as to Lp-PLA2 being a significant predictor of cardiovascular risks. The patent application shows for the first time combining the risks of Lp-PLA2 and CRP to assess the risk of CVD. Figure 7 shows the result of an increased risk for CVD when the risks of Lp-PLA2 and CRP are combined. A patient with elevated levels of both Lp-PLA2 and CRP has a 20% ($1.67/1.38=1.2$) and 40% ($1.67/1.18=1.4$) higher risk of CVD over a patient with elevated levels of either CRP or Lp-PLA2, respectively. Figure 1 shows the result that combining the risks of Lp-PLA2 and CRP indicates patients with elevated levels of both Lp-PLA2 and CRP are less likely to live as long as a patient with low levels of both Lp-PLA2 and CRP or a patient with elevated levels of either Lp-PLA2 and CRP. The difference in the combined risk is statistically significant over the risk of either Lp-PLA2 or CRP alone.

8. In summary, Packard et al. does not teach combining Lp-PLA2 and CRP risks, the combined Lp-PLA2 and CRP risks, or

methods to combine the risks of Lp-PLA2 and CRP to assess the risk of CVD in a patient. The patent application teaches combining Lp-PLA2 and CRP risks, combined Lp-PLA2 and CRP risks, and several methods to combine the risks of Lp-PLA2 and CRP to assess the risk of CVD in a patient. Combining the risks of Lp-PLA2 and CRP to assess the risk of CVD is not obvious since there were conflicting data in literature prior to this invention regarding the usefulness of Lp-PLA2. The statistical significance of combining these markers, as shown in the patent application, could not have been predicted.

I hereby declare that all statements herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful statements and the like so made are punishable by fine or by imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application, any patent issuing there upon, or any patent to which this verified statement is directed.



Robert L. Wolfert

April 17, 2009
Date

A Prospective Evaluation of Lipoprotein-Associated Phospholipase A₂ Levels and the Risk of Future Cardiovascular Events in Women

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OBJECTIVES

We sought to determine prospectively whether lipoprotein-associated phospholipase A₂ (Lp-PLA₂) was a predictor of future cardiovascular risk in women.

BACKGROUND

Inflammatory markers may help predict cardiovascular risk. Lp-PLA₂ levels have recently been hypothesized to be an independent predictor of cardiovascular risk in hypercholesterolemic men.

METHODS

We conducted a prospective, nested case-control study among 28,263 apparently healthy middle-aged women to assess the risk of death from coronary heart disease, non-fatal myocardial infarction, and stroke associated with baseline levels of Lp-PLA₂ over a mean follow-up of three years.

RESULTS

In univariate analysis, mean levels of Lp-PLA₂ correlated strongly with low-density lipoprotein cholesterol ($r = 0.51$; $p = 0.0001$), were lower among women currently using hormone replacement therapy (mean 0.98 mg/l vs. 1.23 mg/l; $p = 0.0001$) and were significantly higher at baseline among cases ($n = 123$) than controls ($n = 123$) (mean 1.20 mg/l vs. 1.05 mg/l; $p = 0.016$). However, the predictive value of Lp-PLA₂ was markedly attenuated after adjustment for these and other cardiovascular risk factors. Specifically, the multivariate relative risks of future cardiovascular events for women in the lowest (referent) to highest quartiles of Lp-PLA₂ were 1.00, 0.75, 0.64 and 1.17, respectively (all p values non-significant). In contrast, the adjusted relative risks of future cardiovascular events for each increasing quartile of C-reactive protein (another marker of low-grade inflammation) were 1.00, 1.78, 2.02 and 4.66, respectively (p -value for trend = 0.002). Inclusion of Lp-PLA₂ levels did not significantly attenuate this latter observation.

CONCLUSIONS

In contrast to prior data among hypodiploidemic men, the current data suggest that Lp-PLA₂ is not a strong predictor of future cardiovascular risk among unselected women. (J Am Coll Cardiol 2001;38:1302-6) © 2001 by the American College of Cardiology

Inflammatory processes play a fundamental role in the pathogenesis of atherosclerosis (1), and several inflammatory biomarkers including high-sensitivity C-reactive protein (CRP), interleukin-6 and soluble intercellular adhesion molecule-1 have been shown to predict future vascular risk (2-11). Recently, lipoprotein-associated phospholipase A₂ (Lp-PLA₂) has been proposed as an inflammatory marker of cardiovascular risk (12-14). This enzyme circulates in the blood in association with low-density lipoprotein (LDL) cholesterol. The synthesis of this enzyme is regulated by inflammatory cytokines (15). Lp-PLA₂ may contribute directly to atherogenesis, by hydrolyzing oxidized phospholipids into pro-atherogenic fragments and by generating lysolecithin, which has pro-inflammatory properties (16).

Alternatively, Lp-PLA₂ may have antithrombotic effects by hydrolyzing platelet-activating factor (14,17).

Recently, Packard and colleagues reported in a cohort of hypercholesterolemic men that increasing plasma levels of Lp-PLA₂ were a strong predictor of risk for incident coronary heart disease (CHD) (13). We sought to test this hypothesis prospectively in a lower-risk population of women.

METHODS

The Women's Health Study (WHS) is an ongoing randomized, double-blind, placebo-controlled trial of aspirin and vitamin E being conducted among women age 45 years and above with no history of cardiovascular disease or cancer (18). Blood samples were collected in EDTA-containing tubes from 28,263 (71%) of these women at baseline and stored in liquid nitrogen until analysis.

Questionnaires are sent to WHS participants to elicit information on cardiovascular risk factors and incident cardiovascular events. For this analysis, cases were defined as study participants who provided a baseline blood sample and who subsequently had a cardiovascular event as defined by death due to CHD, non-fatal myocardial infarction (MI) or stroke. The mean follow-up period was three years.

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Lp-PLA₂ and Future Cardiovascular Risk in Women

Abbreviations and Acronyms

CI	= confidence interval
CHD	= coronary heart disease
CRP	= C-reactive protein
HDL	= high-density lipoprotein
LDL	= low-density lipoprotein
Lp-PLA ₂	= lipoprotein-associated phospholipase A ₂
MI	= myocardial infarction
WHS	= Women's Health Study

For all cases of MI, stroke or death due to CHD, hospital records were obtained and reviewed. Myocardial infarction was confirmed if symptoms met World Health Organization criteria and if the event was associated with diagnostic electrocardiographic changes or elevated cardiac enzymes. Reported stroke was confirmed if the patient had a new neurologic event persisting for more than 24 h; computed tomography scans or magnetic resonance images were available for the majority of women who developed stroke. Death due to CHD was confirmed by review of autopsy reports, death certificates, medical records and circumstances of death.

For each case who provided a baseline blood sample, one control subject matched for age (within one year) and smoking status (current, former or never) was selected from among the remaining study participants who remained free of cardiovascular events and who had also provided a blood sample at baseline. Using these criteria, 123 cases and 123 controls were selected. The cases were composed of 63 women who had a non-fatal MI, 49 women who had a stroke and 11 women who died from CHD.

Baseline plasma samples were thawed and assayed for Lp-PLA₂ with an enzyme-linked immunoassay as previously described (13). Samples were captured with a monoclonal antibody against Lp-PLA₂ and the enzyme identified

with a second monoclonal antibody labeled with biotin and a streptavidin-alkaline phosphatase conjugate. The standard was purified recombinant Lp-PLA₂ and there was no cross-reactivity with other phospholipase A₂ enzymes. C-reactive protein assays were performed using a latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Newark, Delaware) (19). Samples were handled in a blinded fashion throughout the study.

The Student *t* test was used to evaluate differences in means. Because the distribution of CRP was skewed, differences in medians were tested with the rank-sum test. The chi-square statistic was used to compare proportions. Analysis of trends was used to test for any evidence of association between increasing levels of each plasma marker and the risk of future cardiovascular events, after the sample was divided into quartiles according to the distribution of each marker. Pearson's coefficient was used to assess the correlation between Lp-PLA₂ and LDL cholesterol, high-density lipoprotein (HDL) cholesterol and body mass index.

Logistic regression models were used to calculate relative risks and 95% confidence intervals (CIs). In addition to accounting for the variables used for matching (age and smoking status), these models adjusted for random assignment to aspirin or vitamin E. Further analyses were performed that included LDL and HDL cholesterol, body mass index, a history of hypertension, a history of diabetes, a parental history of MI, frequency of exercise and current use of hormone replacement therapy. All *p* values were two-tailed.

RESULTS

The baseline clinical characteristics of the study population are shown in Table 1. As expected, the women who had cardiovascular events had a higher mean body mass index, and were more likely to have a history of hypertension, a

Table 1. Baseline Clinical Characteristics of the Study Population

Characteristic	Women With Cardiovascular Events (Cases) n = 123	Women Free of Cardiovascular Events (Controls) n = 123	p Value*
Mean age (yrs) (\pm SD)	60.2 (\pm 8.3)	60.2 (\pm 8.3)	—
Mean body mass index (kg/m^2) (\pm SD)	27.7 (\pm 5.5)	25.6 (\pm 4.8)	0.002
History of hypertension (%)	58.5	32.8	0.001
History of diabetes (%)	11.4	3.3	0.014
Parental history of myocardial infarction before 60 years (%)	22.8	11.2	0.02
Smoking status (%)			—
Current	28.5	28.5	
Former	32.5	32.5	
Never	39.0	39.0	
Current use of hormone replacement therapy (%)	43.9	41.5	0.7
Lp-PLA ₂ (mg/l) mean (\pm SD)	1.20 (\pm 0.56)	1.05 (\pm 0.41)	0.016
CRP (mg/dl) median (interquartile range)	0.51 (0.26–0.85)	0.26 (0.12–0.49)	0.0001

**p* values were not calculated for variables used in matching of case and control subjects, because the distribution of these variables was identical between the two groups.

Lp-PLA₂ = lipoprotein-associated phospholipase A₂; SD = standard deviation.

Table 2. Crude and Adjusted Relative Risks of Cardiovascular Events According to Quartile of Plasma Level of Lp-PLA₂

	Quartile of Plasma Level of Lp-PLA ₂				p Value for Trend
	1 (lowest)	2	3	4 (highest)	
Plasma level (mg/l)	=0.77	0.78-1.01	1.02-1.29	≥1.30	
Crude Analysis*					
Relative risk	1	0.90 (0.43-1.85)	0.69 (0.32-1.47)	1.73 (0.87-3.44)	0.14
(95% CI)					
p value		0.76	0.33	0.12	
Adjusted for LDL and HDL†					
Relative risk	1	0.72 (0.34-1.51)	0.50 (0.22-1.13)	1.08 (0.48-2.43)	0.8
(95% CI)					
p value		0.38	0.10	0.86	
Adjusted for LDL, HDL and other risk factors‡					
Relative risk	1	0.75 (0.31-1.79)	0.64 (0.25-1.63)	1.17 (0.45-3.05)	0.8
(95% CI)					
p value		0.52	0.34	0.75	

*All models were adjusted for random assignment to aspirin or vitamin E. †These models were also adjusted for LDL and HDL cholesterol. ‡These models were also adjusted for the following risk factors: LDL and HDL cholesterol, body mass index, a history of hypertension, a history of diabetes, a parental history of myocardial infarction, frequency of exercise and current use of hormone replacement therapy.

CI = confidence interval; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Lp-PLA₂ = lipoprotein-associated phospholipase A₂; p values were calculated by logistic regression analysis.

history of diabetes, or a parental history of early MI (before the age of 60 years) than women free from cardiovascular events. Because of matching, cases and controls were almost identical with respect to age and smoking status.

In univariate analyses, baseline levels of Lp-PLA₂ were higher among cases than controls (mean 1.20 mg/l vs. 1.05 mg/l; $p = 0.016$) (Table 1). However, levels of Lp-PLA₂ were also highly correlated with LDL cholesterol ($r = 0.51$, $p = 0.0001$), as well as body mass index ($r = 0.22$, $p = 0.0007$) and HDL cholesterol ($r = -0.34$, $p = 0.0001$). Thus as shown in Table 2, after adjustment for these and other cardiovascular risk factors, there was little evidence of association between Lp-PLA₂ and future cardiovascular risk. Specifically, the adjusted relative risks from lowest (referent) to highest quartiles of Lp-PLA₂ at baseline were 1.00, 0.75, 0.64 and 1.17 (p -value for trend = 0.8). In comparison, the relative risks associated with each increasing quartile of CRP, after adjustment for both lipid levels and other traditional risk factors, were 1.00, 1.78, 2.02 and 4.66 respectively (p -value for trend = 0.002) (Fig. 1). As shown in Table 3, the adjusted risk of future cardiovascular events increased by 62% for each quartile increase in CRP ($p = 0.002$), whereas the risk increased by 5% for each quartile of Lp-PLA₂, an effect that was not statistically significant ($p = 0.8$). These results were essentially unchanged when both Lp-PLA₂ and CRP were included in the same model.

To determine whether there was a threshold effect above which Lp-PLA₂ levels conferred an increased risk, we performed a post-hoc analysis in which we calculated the relative risk of future cardiovascular events at different cutoffs of Lp-PLA₂ ranging from the 50th to the 95th percentile. In this post-hoc analysis, women with levels above the 95th percentile had a significantly increased risk in univariate analyses (relative risk = 2.67, 95% CI 1.106 to 6.69; $p = 0.04$). Again, however, even among this small

subgroup of women with the very highest baseline levels of Lp-PLA₂, this effect was attenuated in multivariate analysis (relative risk = 2.08, 95% CI 0.69 to 6.30; $p = 0.2$).

In subgroup analyses restricted to stroke as a separate endpoint, for each quartile increase of Lp-PLA₂ the crude relative risk of stroke increased by 6% (95% CI -21% to +41%; $p = 0.7$), whereas for each quartile increase of CRP the crude relative risk of stroke increased by 78% (95% CI +28% to +248%; $p = 0.0006$).

Lp-PLA₂ levels were higher among women currently not taking hormone replacement therapy ($n = 141$) than among women taking hormone replacement therapy ($n = 105$) ($1.23 \text{ mg/l} \pm \text{vs. } 0.98 \text{ mg/l} \pm 0.47$; $p = 0.0001$). This finding is consistent with data from animal models suggesting that estrogen decreases plasma Lp-PLA₂ activity (20,21). Thus we examined for evidence of effect modification according to current use of hormone replacement therapy. In analyses restricted to women not currently taking hormone replacement therapy, the unadjusted relative risk of future cardiovascular events increased by 29% with each quartile increase of Lp-PLA₂ (95% CI -4% to +72%; $p = 0.09$). This effect was attenuated in adjusted analyses, where the relative risk increased by 7% for each quartile increase of Lp-PLA₂ (95% CI -29% to +62%; $p = 0.7$). Among women currently taking hormone replacement therapy, the relative risk of future cardiovascular events for each quartile increase of Lp-PLA₂ increased by 10% (95% CI -21% to +52%; $p = 0.6$) in crude analysis and 3% (95% CI -37% to +66%; $p = 0.9$) in adjusted analysis.

DISCUSSION

In this prospective study of healthy middle-aged women, plasma levels of Lp-PLA₂ were higher among women who

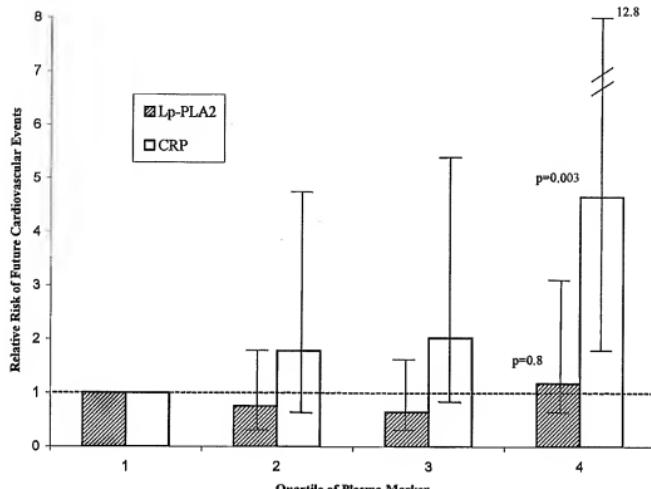


Figure 1. Adjusted relative risks of cardiovascular events according to increasing quartiles of lipoprotein-associated phospholipase A₂ (Lp-PLA₂) and C-reactive protein (CRP) compared to the lowest quartile. The error bars indicate the 95% CIs. The p values are for the highest quartile of plasma marker compared to the lowest quartile. These models were adjusted for random assignment to aspirin and vitamin E and for the following risk factors: low-density lipoprotein and high-density lipoprotein cholesterol, body mass index, a history of hypertension, a history of diabetes, a parental history of myocardial infarction, frequency of exercise and current use of hormone replacement therapy.

subsequently developed cardiovascular events than in those who remained free of cardiovascular events. However, this effect was minimal and no longer statistically significant in analyses adjusting for traditional cardiovascular risk factors. In contrast, among the same women another marker of inflammation, CRP, was a significant predictor in both univariate and multivariate analyses.

Our univariate results are consistent with the finding of Packard and colleagues (13) that Lp-PLA₂ levels are somewhat higher at baseline among patients who subsequently have cardiovascular events than in those who remain free of cardiovascular disease. These data also confirm prior observations that Lp-PLA₂ levels are highly correlated with LDL cholesterol levels (13). However, the current data do not

find an important role for Lp-PLA₂ as an independent predictor of future cardiovascular risk in women.

There are several potential explanations for these observed differences. First, our study evaluated women rather than men, and thus it is theoretically possible that gender differences exist for Lp-PLA₂. The use of hormone replacement therapy may have affected our results, although prevalence of hormone replacement therapy did not differ between cases and controls, we controlled for use of hormone replacement in our adjusted analysis, and no significant difference was observed in analyses stratified by hormone replacement therapy use.

Second, in contrast to the hypothesis-generating study from Packard and colleagues that evaluated hypercholester-

Table 3. Crude and Adjusted Relative Risks of Cardiovascular Events Associated With a One-Quartile Increase in the Concentration of Lp-PLA₂ and CRP

Variable	Unadjusted* Relative Risk (95% CI)	p Value	Adjusted† Relative Risk (95% CI)	p Value
Lp-PLA ₂	1.18 (0.95–1.46)	0.14	1.05 (0.78–1.42)	0.8
CRP	1.82 (1.42–2.33)	0.0001	1.62 (1.19–2.20)	0.002

*All models were adjusted for random assignment to aspirin and vitamin E. †These models were also adjusted for the following risk factors: low-density lipoprotein and high-density lipoprotein cholesterol, body mass index, a history of hypertension, a history of diabetes, a parental history of myocardial infarction, frequency of exercise and current use of hormone replacement therapy.

CI = confidence interval; CRP = C-reactive protein; Lp-PLA₂ = lipoprotein-associated phospholipase A₂.

olemic individuals (13), our study evaluated a much broader cohort with lipid levels representative of the general population. Thus, as both our study and that of Packard demonstrate strong correlation between Lp-PLA₂ and LDL (as well as inverse correlation with HDL), it is possible that any true predictive value of Lp-PLA₂ may be limited to those with overt hyperlipidemia. Indeed, the strong correlation between Lp-PLA₂ and LDL likely explains why the distribution of Lp-PLA₂ values in our study is lower than that observed in the Packard data despite using an identical assay.

Third, our study involved a smaller sample size than the West of Scotland report; nonetheless previous studies of similar size to ours have found other novel plasma markers of inflammation to be significant predictors of future cardiovascular risk (10,22). Furthermore, in the present study, baseline levels of CRP were a significant predictor of future risk, indicating that this sample was of adequate size for another marker of inflammation.

Finally, the randomized use of aspirin in the present study, or pravastatin in the West of Scotland study, may have affected the results. However, our analyses were adjusted for randomized use of aspirin and vitamin E, so we do not believe this had an important effect on our results.

Conclusions. In the current data, women with the very highest levels of Lp-PLA₂ at baseline (>95th percentile) did appear to have an increased risk of future cardiovascular events. Thus, although these data do not confirm a role for Lp-PLA₂ as a potential screening test for atherosclerotic risk in a general population, they do support further research regarding this unique phospholipase in sub-populations selected for more traditional risk factors.

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